The Young Notochord can Induce Somite Genesis by Means of Diffusible Substances in the Chick

In this transfilter experiments, COOPER[1] has recently reported that the differentiated notochord of the chick embryo elicits chondrogenesis in the somitic mesoblast. As far as earlier stages are concerned, the notochord can promote somite formation in amphibians (YAMADA[2]) as well as in birds (NICOLET[3]). In previous experiments (NICOLET[3]), the induced somites were closely associated with the notochord implant. It suggests that diffusible products elaborated by the young notochord may also be responsible for this induction. We are trying at present to test this assumption.

Chick blastoderms were transplanted in culture in vitro at the head process stage (GALLERA and NICOLET[4]). As seen in Figure 1, two types of operations were performed. In the first series, a large square of the germ wall was extirpated in the anterior region of the area opaca, a short piece of notochord including the chorda-bulb was cut out, explanted in this place and covered by a square of millipore filter (square side 1.2 mm, thickness 25 μm and pore size 0.45 μm). As reacting fragment, a primitive streak explant was excised 0.8 mm behind the node since the streak mesoblast is incapable of self differentiation into somites at this level (NICOLET[3]). As one guessed the contours of the underlying notochord piece, we were able to put the explant just over it. Lastly, a millipore fragment was applied over the embryonic axis to prevent the enlargement of the injuries. As a second series, control experiments were devised. They simply consisted in explanting a primitive streak fragment of the same level on the millipore surface to ascertain that such culture conditions did not alone alter their self differentiation potencies. 11 experiments and 8 controls were carried out. After 24 h of culture, the blastoderms were fixed and the results were analyzed on serial sections of 8 μm.

As a whole, the primitive streak fragment behaved similarly in both series. It stuck more or less tightly to the filter so that its peripheral expansion was reduced. Later on, it assumed a lenticular shape and never extended beyond the filter margin (Figure 2). Examination of sections shows that the mesoblast segregation was often deficient. Nevertheless, explants often yielded lateral plate in the middle and blood islands all around. The germ wall actively invaced the millipore filter, especially its upper surface, and covered the dorsal side of the explant more or less completely (Figure 2). Below, the notochord piece gave rise to an elongated rod. In the experimental series, 8 cases out of 11 gave a positive response: 2 to 6 well segmented somites differentiated just over the notochord. On the contrary, no response was recorded in controls. In their general aspects, these somites looked like young somites shortly after their segmentation (Figure 3).

The present results allow us to conclude that the young notochord must be held responsible for the somite formation which occured in the explants, since its presence is

[1] G. W. COOPER, Devl. Biol. *12*, 185 (1965).
[2] T. YAMADA, Okajimas Folia anat. jap. *19*, 131 (1940).
[3] G. NICOLET, J. Embryol. exp. Morph. *24*, 467 (1970).
[4] J. GALLERA and G. NICOLET, Experientia *17*, 134 (1961).

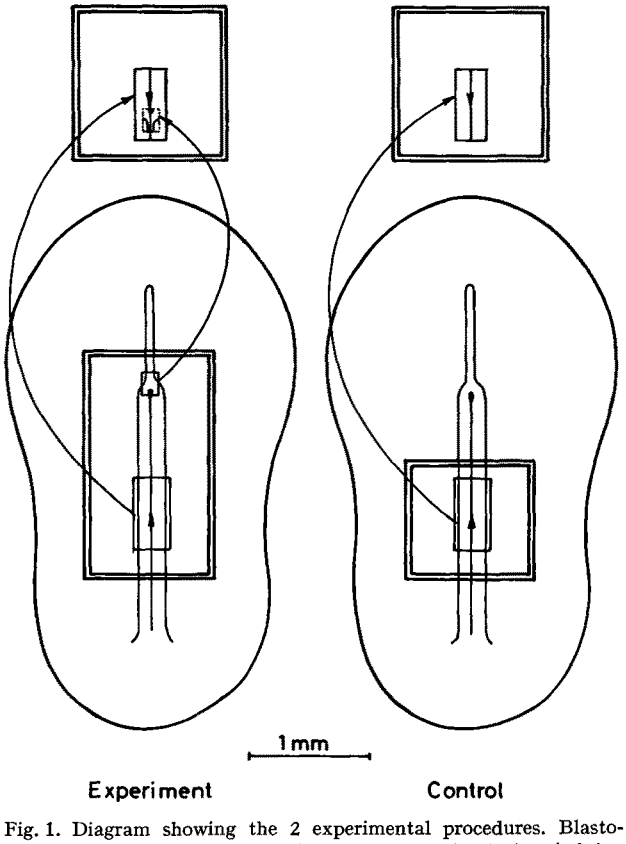

Fig. 1. Diagram showing the 2 experimental procedures. Blastoderms are seen from the ventral side. The notochord piece is lying beneath the filter (stippled line). Double lined areas designate the fragments of millipore filter. See text for detail comments.

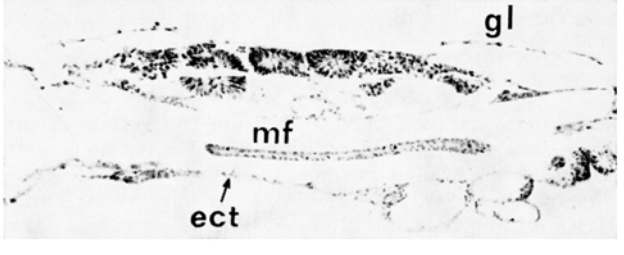

Fig. 2. This section photographed at a low magnification (×160) shows that the primitive explant is covered by a thin layer of germ wall and does not extend beyond the filter margin. Induced somites differentiate just over the notochord, which is cut longitudinally. gl, germ wall layer; mf, millipore filter and ect, ectoblast.

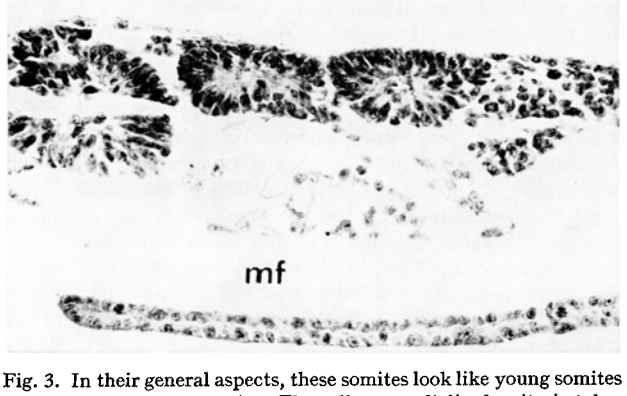

Fig. 3. In their general aspects, these somites look like young somites just after their segmentation. The cells are radialized, mitosis takes place inside the central matrix and the nuclei tend towards the basal pole of each cell (×380). mf, millipore filter.

absolutely required. They show that its inductive action has its most efficient effect on the mesoblast lying just over it. Furthermore, they provide clear evidence that it induces somites through the medium of diffusible substances, since it has been formally demonstrated that the porous membrane filter prevents any direct contact between the 2 interacting components (GROBSTEIN[5], NYHOLM et al.[6], GALLERA et al.[7]). If these results are compared to those which were obtained in other systems, we find that the somite genesis recalls in some way what several authors, namely SAXEN et al.[8], have observed during the kidney tubulogenesis. In both cases, the cells indeed aggregate when the diffusible inductive factors have modified their behaviour and their mutual affinity[9].

Résumé. L'utilisation de fragments de filtre millipore, intercalés entre un fragment de ligne primitive et un explantat de jeune chorde, nous permet de démontrer présentement que l'induction des somites par la chorde s'opère par le truchement de substances diffusibles chez les oiseaux.

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[5] C. GROBSTEIN, Expl. Cell. Res. *13*, 575 (1957).
[6] M. NYHOLM, L. SAXEN, S. TOIVONEN and T. VAINIO, Expl. Cell. Res. *28*, 209 (1962).
[7] J. GALLERA, G. NICOLET and M. BAUMANN, J. Embryol. exp. Morph. *19*, 439 (1968).
[8] L. SAXEN, O. KOSKIMIES, A. LAHTI, H. MIETTINEN, J. RAPOLA and J. WARTIOVAARA, Adv. Morphogen. *7*, 251 (1968).
[9] This work was generously supported by the Fonds National de la Recherche Scientifique, Bern (Switzerland).

Succinic Dehydrogenase in the Cnidoblast of Hydra and its Role in the Discharge of Nematocyst

There are a large number of light and electron microscopic observations[1-7] concerned with the structure and discharge of the stenotele of hydra. As far as the source of energy for firing the nematocyst is concerned, the electron microscopic studies present very little evidence. Apart from these considerations, no histochemical studies have been reported on the localization and distribution pattern of oxidative enzymes in the cnidoblast of hydra. Among histochemically detectable enzymes of the Krebs cycle, succinic dehydrogenase (SDH) was found to be the most distinctive in preliminary studies. The present note, therefore, deals with the histochemical localization of SDH in cnidoblast of hydra and its role in the discharge of nematocyst.

Fresh specimens of hydra were collected locally from the ponds in the campus. The tentacles were cut from the living specimens, teased out a little and then transferred directly into the incubation medium for SDH. The composition of the medium was as described by PEARSE[8]. Nitro blue tetrazolium was used as the electron acceptor and the incubation medium was also supplemented with phenazine methosulfate. The material was incubated in toto for 20 min, washed in distilled water, fixed in 10% solution of buffered neutral formalin for 3 min and then after washing mounted in glycerol jelly.

Microscopic observations revealed the presence of sharp bluish diformazan spherical granules (Figure), representing the approximate sites of mitochondria, as it is known that SDH is exclusively confined to mitochondria. Since succinate is considered to be major metabolite of the Krebs cycle, it is obvious that there is a definite source of energy supply through the operation of the Krebs cycle in mitochondria, which lie in the neighbourhood of the fine fibrils or tubules reported earlier[7,9]. The presence of these fine fibrils or tubules, showing a similarity to the basic contractile structures, along with high SDH activity in the cnidoblast, suggest a definite role of these tubules in contraction, thereby bringing about a forceful discharge of the nematocyst[10].

Zusammenfassung. Nachweis von Succinodehydrogenase in unmittelbarer Nähe der Nesselkapseln bei Hydra.

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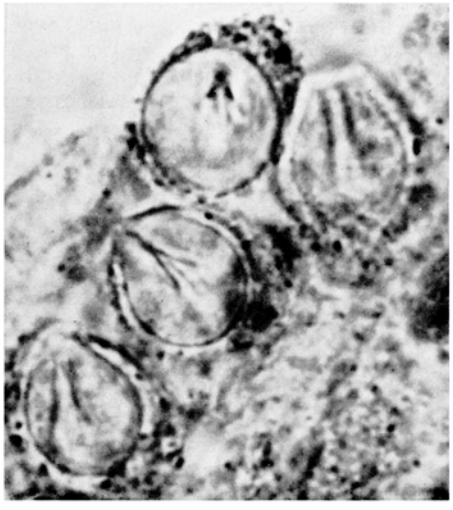

Photomicrograph showing histochemical localization of succinic dehydrogenase in the cnidoblast of hydra. Conditions as described in the text. Darkly stained spherical granules represent the sites of the enzyme activity. ×1000.

[1] L. E. R. PICKEN, Q. Jl microsc. Sci. *94*, 203 (1953).
[2] E. A. ROBSON, Q. Jl microsc. Sci. *94*, 229 (1953).
[3] G. B. CHAPMAN and L. G. TILNEY, J. biophys. biochem. Cytol. *5*, 69 (1959).
[4] G. B. CHAPMAN and L. G. TILNEY, J. biophys. biochem. Cytol. *5*, 79 (1959).
[5] G. B. CHAPMAN, in *The Biology of Hydra and Some Other Coelenterates* (Eds. H. M. LENHOFF and W. F. LOOMIS; University of Miami Press, Florida 1961), p. 131.
[6] D. B. SLAUTTERBACK, in *The Biology of Hydra and of Some Other Coelenterates* (Eds. H. M. LENHOFF and W. F. LOOMIS; University of Miami Press, Florida 1961), p. 77.
[7] D. B. SLAUTTERBACK, J. Cell Biol. *18*, 367 (1963).
[8] A. G. E. PEARSE, *Theoretical and Applied Histochemistry*, 2nd edn (J. and A. Churchill, Ltd., London 1961), p. 910.
[9] C. F. T. MATTERN, H. D. PARK and W. A. DANIEL, J. Cell Biol. *27*, 621 (1965).
[10] Grateful thanks are due to Prof. B. R. SESHACHAR for the interest and the necessary facilities provided for this work.